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Journal of Chromatography B, 724 (1999) 365–371

JOURNAL OF CHROMATOGRAPHY B

Capillary electrophoresis of ^{99m}technetium radiopharmaceuticals

R. Jankowsky*, B. Noll, B. Johannsen

Institut für Bioanorganische und Radiopharmazeutische Chemie, Forschungszentrum Rossendorf, Postfach 510119, D-01314 Dresden, Germany

Received 9 November 1998; received in revised form 7 December 1998; accepted 7 December 1998

Abstract

Diagnostically used ^{99m}Tc kit radiopharmaceuticals were analyzed using capillary zone electrophoresis with radioactivity detection: ^{99m}Tc-bis(bis(2-ethyloxyethyl)phosphino)ethane (^{99m}Tc-Myoview, ^{99m}Tc-Tetrofosmin), ^{99m}Tc-*trans*(1,2-bis(dehydro-2,2,5,5,-tetramethyl-3-furanone-4-methylene-amino)ethane)-*tris*(3-methoxy-1-propyl)phosphine) (^{99m}Tc-Technescan Q12, ^{99m}Tc-Furifosmin), ^{99m}Tc-methoxyisobutylisonitrile (^{99m}Tc-MIBI), ^{99m}Tc-L,L-ethylenecysteine diethylester dimer (^{99m}Tc-ECD), ^{99m}Tc-d,l-hexamethylene propyleneamine oxime (^{99m}Tc-HMPAO), ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA), ^{99m}Tc-ethylene hepatobiliary iminodiacetic acid (^{99m}Tc-EHIDA), ^{99m}Tc-L,L-ethylenecysteine dimer (^{99m}Tc-EC), ^{99m}Tc-mercaptoacetylglycylglycylglycine (^{99m}Tc-MAG_3), ^{99m}Tc-dimercaptosuccinic acid (^{99m}Tc-DMSA), ^{99m}Tc-methylene diphosphonate (^{99m}Tc-MDP) and $^{99m}NaTcO_4$. A pressure-driven capillary zone electrophoresis was employed to detect small anions of high electrophoretic mobility and cations within one run. Effective ^{99m}Tc complex charges could be determined by a neutral internal standard. All complexes showed the expected electrophoretic behaviours in view of their charges. Pure products were obtained for the majority of the studied complexes. In the case of ^{99m}Tc-Q12, ^{99m}Tc-EHIDA and ^{99m}Tc-MDP, complex mixtures were detected. The high potential of CE for the analysis of ^{99m}Tc radiopharmaceuticals could be shown.

Keywords: Radiopharmaceuticals; ^{99m}Technetium

1. Introduction

Nuclear medicine contributes decisively to modern medical diagnostics. It employs the radiotracer concept which is basing on the accumulation and detection of radioactive radiation emitted by the radiotracer in patients. By usage of radiotracers, several metabolic and pathological processes may be visualized. Localization of radiotracers is accomplished by detection devices (cameras), which are used to produce three-dimensional image of the radioactivity accumulation in the patient's body. According to the applied technique, different kinds of radioactive nuclides are used for the radiotracers. In positron emission tomography (PET), positron emitting nuclides such as ^{18}F or ^{11}C are used. For single photon emission computer tomography (SPECT), γ-ray emitting nuclides are employed. The majority of nuclear medical diagnostics is carried out with the SPECT technique using the nuclide ^{99m}Tc [1–6]. It is the radionuclide of choice in daily clinical routine, since it exhibits nearly ideal radiation properties (γ-energy 140 keV, half-life 6 h) and is readily available through a ^{99}Mo/^{99m}Tc generator [7]. Within the generator, ^{99m}Tc is generated by the radioactive decay of the mother nuclide ^{99}Mo which

*Corresponding author.

0378-4347/99/$ – see front matter
PII: S0378-4347(98)00577-5

is immobilized as sodium permolybdate $NaMoO_4$ onto a column. Elution of the generator delivers the desired decay product $Na^{99m}TcO_4$ in saline [7]. The pertechnetate solution is used to prepare the desired radiopharmaceuticals. Such radiopharmaceuticals are conveniently prepared by kits that contain all precursor substances and reagents such as a ligand for complexation of the ^{99m}Tc, an agent for reducing $^{99m}TcO_4^-$, in certain cases transchelation agents and other ingredients. The obtained radiopharmaceuticals may contain ^{99m}Tc with various oxidation states [1,3–6].

Usually, the quality of the radiopharmaceutical preparations is checked by thin layer chromatography (TLC) coupled with a radioactivity measuring device. In radiopharmaceutical research, the radio-HPLC basing on reversed-phase columns is currently the sophisticated method to analyze ^{99m}Tc preparations. It does, however, suffer from some undesired effects. Depending on the nature of the ^{99m}Tc agents, irreversible adsorption to the stationary phase or very long equilibration periods of the columns after each run may be required. Another aspect is the production of relative large amounts of radioactive eluent waste. Consequently, alternative approaches for quality control and investigation of complex reaction mixtures are required. A promising possibility is to separate them by electrophoresis, since the complexation of the ^{99m}Tc central atom leads to a high charge density at the metal core.

In the present work, capillary zone electrophoresis (CZE) was applied to the separation of radiopharmaceutically relevant ^{99m}Tc preparations. The CZE techniques is well-established for chemical and biochemical problems and is characterized by short analyzing times and very efficient separations [8]. In general, detection is performed by built-in UV–VIS detection systems. In the case of ^{99m}Tc complexes, the metal concentration is between 10^{-8} and 10^{-12} *M*, thus a UV–VIS detection is not applicable. Instead of this, a radioactivity detection employing the γ-ray emission of the radioactive nuclides is necessary. While radioactivity detection systems are routinely used in HPLC analysis, radioactivity detection sensitivity in capillary electrophoresis renders this more difficult because of the very small injected sample amounts. This issue has been addressed recently [9–16]. First CZE studies were described for ^{99m}Tc-containing radiopharmaceuticals [13,14].

Here we present CZE results of the quality control of a variety of commonly used ^{99m}Tc radiopharmaceuticals [1,3–6]. A γ-sensitive radioactivity detector coupled to a commercially available capillary electrophoresis device was employed. Since small anions with very high electrophoretic mobility, such as $^{99m}TcO_4^-$, are poorly detectable by conventional CZE means [13,14], pressure-driven CZE analysis was applied.

2. Experimental

2.1. ^{99m}Tc kit preparation

Kits for the preparation of ^{99m}Tc-MAG_3, ^{99m}Tc-DTPA, ^{99m}Tc-DMSA, ^{99m}Tc-HMPAO, ^{99m}Tc-EHIDA and ^{99m}Tc-MDP were gifts from ROTOP Nuclear Engineering and Analytics, Dresden, Germany. ^{99m}Tc-EC kits were provided by the Department of Radiopharmaceuticals Production, Medical University Foundation, Lodz, Poland. Technescan Q12 kits were obtained from Mallinckrodt Medical B.V. Petten, The Netherlands. They were prepared with $Na^{99m}TcO_4$ generator eluate from a UltraTechnekow generator (Mallinckrodt Medical). Ready kit preparations of ^{99m}Tc-ECD (DuPont, Bad Homburg, Germany), ^{99m}Tc-Myoview (Amersham International, Buckinghamshire, UK) and ^{99m}Tc-MIBI (DuPont, Bad Homburg, Germany) were gifts from the Clinic for Nuclear Medicine at the University Hospital of the Dresden University of Technology, Dresden, Germany. The samples were of 20 and 30 MBq per 100 μl solution.

2.2. Capillary electrophoresis of ^{99m}Tc radiopharmaceuticals

Measurements were performed using a Hewlett-Packard ^{3D}CE device equipped with a diode-array detection system (DAD). A non-coated fused-silica capillary with an inner diameter of 75 μm (Hewlett-Packard, Waldbronn, Germany), a total length of 72 cm and an effective length up to the UV–VIS detection cell of 64 cm was employed. For radioactivity detection, a Steffi γ-detector with a NaI crystal scintillation probe (Raytest, Straubenhardt, Germany) was used, as described in [17]. The effective

length from the capillary inlet to the radioactivity detector was 47.4 cm.

As background electrolyte, a phosphate buffer of pH 7.4 (0.02 *M*) was used. Prior to each run, buffer vials were replenished to yield equal filling levels. For capillary conditioning, the capillary was rinsed for 2 min with 0.1 *M* NaOH and subsequently for 2 min with the running buffer. Injection was performed by hydrodynamic injection (3 s at 50 mbar analyte, 1 s at 50 mbar running buffer). The electric field was chosen to be positive, i.e. the capillary inlet electrode represented the anode. Thus, cations were eluted prior to neutral and anionic compounds. The separation voltage was 20 kV for all runs. The capillary was thermostated to 25°C during runs. Each sample was spiked with acetone, which served as a neutral marker and was detected by the DAD at 254 nm. Since the DAD was positioned at the 1.35-fold of the effective length of the radioactivity detector, the obtained migration times for the radioactivity signals were multiplied by the factor 1.35 to conclude the ^{99m}Tc complex charges.

Due to the very high anionic electrophoretic mobility of ^{99m}Tc-DMSA, ^{99m}Tc-MAG_3, ^{99m}Tc-MDP and $^{99m}TcO_4^-$, the generated electroosmotic flow inside the capillary was not sufficiently fast to elute them. Therefore, the flow inside the capillary was fastened by applying an external air pressure of 50 mbar onto the capillary inlet vial during runs [18]. This pressure was realized by the built-in air pump of the capillary electrophoresis device.

3. Results and discussion

In conventional CZE, the detection of complex cations and anions with high electrophoretic mobilities within a single separation run is difficult. The $^{99m}TcO_4^-$ ion (pertechnetate) as one of the possible radiochemical impurities can be detected by the application of a negative electric field [13,14], but that prevents the analysis of fast cationic species in the radiopharmaceutical preparations. In contrast, cations may be detected at positive fields where no analysis of $^{99m}TcO_4^-$ is possible. This problem could be overcome by the pressure-driven CZE, i.e. a positive electric field is applied while an external air pressure is given onto the capillary inlet vial during the separation run [16]. By this means, the detection of cationic complexes as well as $^{99m}TcO_4^-$ or other highly-charged anionic complexes within a single run became possible. Furthermore, by covering the entire electrophoretic mobility range, the existence of non-detected impurities is not probable. In Fig. 1, the electropherogram for a pressure-driven CZE run of $^{99m}TcO_4^-$ is presented. It shows a migration time of 4.73 min. Acetone was detected at 1.59 min by the DAD. This value was multiplied by the lengths ratio of the DAD and the radioactivity detector (1.35) and yielded the corrected acetone migration time for the radioactivity detector (dashed line in Fig. 1).

It was found that the complex structure had a significant influence on the wall adsorption in the capillary. This was observed by increased detector background levels after runs. However, it rapidly decreased by rinsing the capillary with sodium hydroxide.

3.1. Cationic ^{99m}Tc radiopharmaceuticals

Cationic ^{99m}Tc radiopharmaceuticals were analyzed by conventional CZE means. In Fig. 2, the electropherograms of ^{99m}Tc-Myoview (^{99m}Tc-Tetrofosmin), ^{99m}Tc-Q12 (^{99m}Tc-Furifosmin) and ^{99m}Tc-MIBI are presented.

Using the conventional CZE method, acetone as the neutral marker was detected at 2.86 min. Thus, the detected single peak for ^{99m}Tc-Myoview can be assigned as cationic. This is in accordance with the proposed structure. Although it is not evident, the

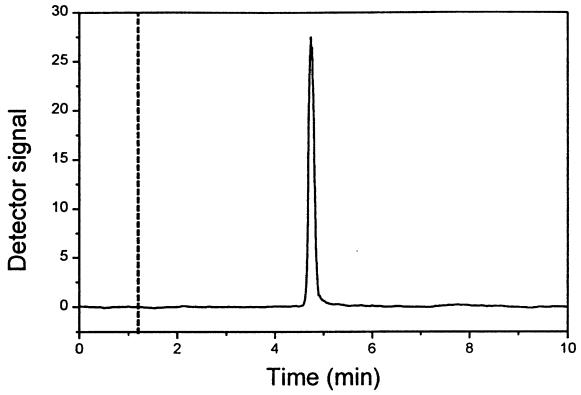

Fig. 1. Electropherogram of $^{99m}TcO_4^-$; the dashed line shows the corrected acetone migration time.

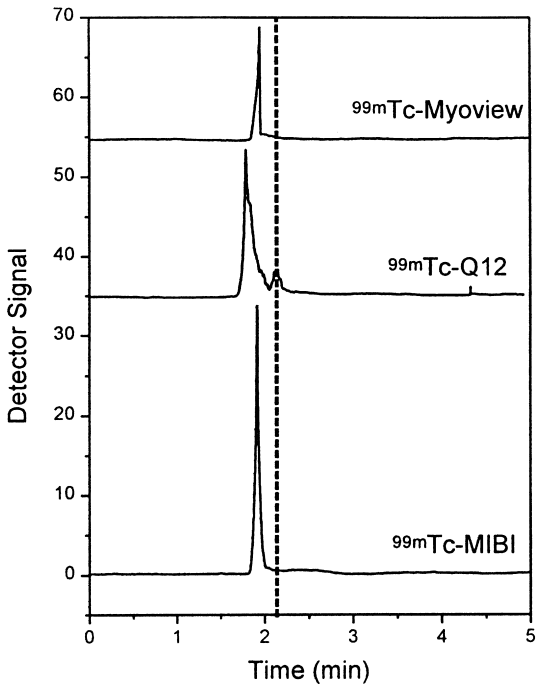

Fig. 2. Electropherograms of cationic ^{99m}Tc radiopharmaceuticals; the dashed line shows the corrected acetone migration time.

occurrence of a single radioactivity peak hints at the radiochemical purity of the complex. In case of the ^{99m}Tc-Q12 complex, multiple products were detected. The main product, which can be considered as cationic, seems to be the desired complex. However, this peak shows a significant tailing giving rise to the assumption that additional cationic compounds are existent. An additional compound occurs at 2.12 min which is considered to be neutral. The kit preparation of ^{99m}Tc-MIBI exhibits a single cationic peak showing that the obtained product is probably uniform. Since the separation in CZE is based on the ratio of absolute charge and hydrated diameter of the analyte, an approximate assessment of the molecular sizes can be made if an effective complex charge of +1 is assumed for all complexes. ^{99m}Tc-Q12 exhibits the highest electrophoretic mobility, followed by ^{99m}Tc-MIBI and ^{99m}Tc-Myoview. This sequence corresponds to an increase of the hydrated complex diameter and thus molecular size increases. For all three cationic radiopharmaceuticals, no free $^{99m}TcO_4^-$ was found in the kit preparations by pressure-driven CZE.

3.2. Neutral ^{99m}Tc radiopharmaceuticals

Neutral complexes were studied using the conventional CZE without application of an external pressure during runs. The electropherograms obtained for ^{99m}Tc-HMPAO and ^{99m}Tc-ECD are depicted in Fig. 3.

The detection times of both complexes are identical and indicate their neutrality. Since it is known that non-stabilized ^{99m}Tc-HMPAO forms a secondary complex after several hours at room temperature [3], another CZE run was performed 2 h after kit reconstitution. Surprisingly, no additional radioactive products occurred in the electropherogram showing that the secondary complex, if formed, is neutral. As expected, the ^{99m}Tc-ECD complex is found to be neutral which is compatible to its assumed complex

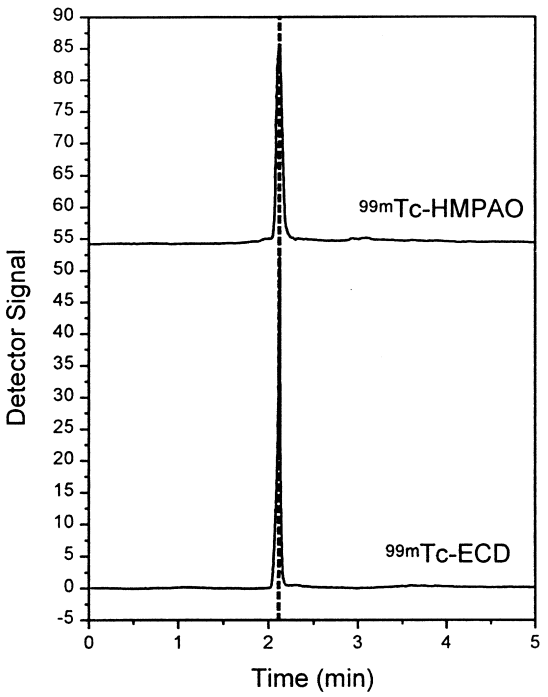

Fig. 3. Electropherograms of neutral ^{99m}Tc complexes; the dashed line shows the corrected acetone migration time.

structure. No free $^{99m}TcO_4^-$ could be found in the analyte solutions.

3.3. Anionic ^{99m}Tc radiopharmaceuticals

In Fig. 4, the electropherograms for ^{99m}Tc-EC, ^{99m}Tc-DTPA and ^{99m}Tc-EHIDA are presented. They were recorded using the conventional CZE mode.

Single peaks were obtained for ^{99m}Tc-EC and ^{99m}Tc-DTPA suggesting that the preparations gave high radiochemical purities. Their different detection times result from the differences in their effective complex charges and their ligand structures. Both complexes are anionic which was expected from their molecular structures. ^{99m}Tc-EC is very similar to the ^{99m}Tc-ECD complex with the ester groups saponified and being deprotonated at the buffer pH. This causes the relative high electrophoretic mobility compared to the other complexes. The narrow single peak detected in the electropherogram shows the uniformity of the complex due to a very stable complex constitution. ^{99m}Tc-DTPA shows a lower electrophoretic mobility as resulting from the lower charge density. In the case of ^{99m}Tc-EHIDA, several anionic products were detected. This suggests that no uniform complex is formed by the kit preparation. All formed compounds are anionic.

The ^{99m}Tc-MAG_3, ^{99m}Tc-DMSA and ^{99m}Tc-MDP complexes were not detectable in conventional CZE mode. Therefore, the pressure-driven CZE was applied to study the kit preparations. Fig. 5 shows the corresponding electropherograms for these complexes.

The lack of detectability of the complexes in conventional CZE mode results from their very high charge density leading to high electrophoretic mobilities. ^{99m}Tc-MAG_3 possesses an effective charge of -2 at physiological pH [1,3]. There is one negative charge at the metal core and another arising from the deprotonation of the free ligand carboxyl group. Since the ligand is wrapped very tightly

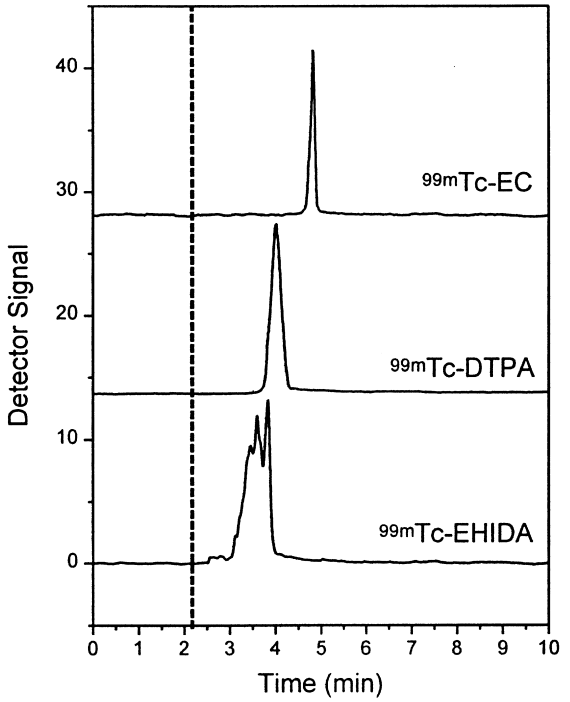

Fig. 4. Electropherograms of anionic ^{99m}Tc radiopharmaceuticals, recorded using the conventional CZE mode; the dashed line shows the corrected acetone migration time.

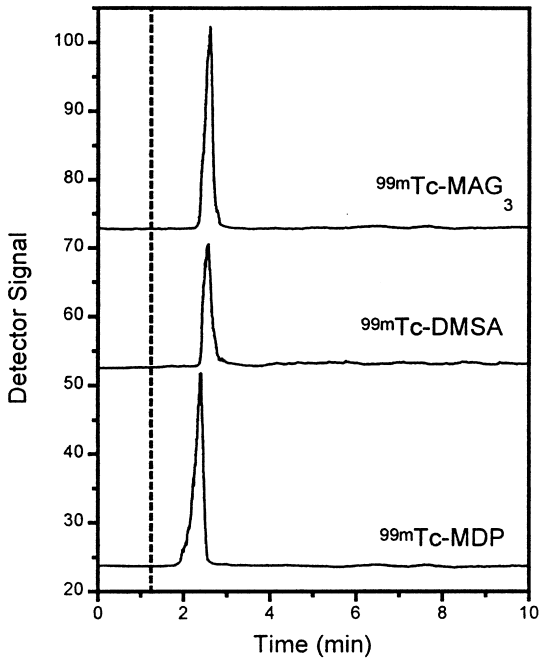

Fig. 5. Electropherograms of anionic ^{99m}Tc radiopharmaceuticals, recorded using the pressure-driven CZE mode; the dashed line shows the corrected acetone migration time.

around the metal, the absolute molecular size appears to be rather small. In combination with a double anionic complex charge, the electrophoretic mobility is thus very high. The electrophoretic mobility of ^{99m}Tc-DMSA complex is comparable to ^{99m}Tc-MAG_3. The more voluminous ligand arrangement is connected with an effective complex charge of -5 at physiological pH [1,3].

The ^{99m}Tc-MDP complex potentially possesses several deprotonated groups at physiological pH probably causing the high electrophoretic mobility of the complex. Compared to ^{99m}Tc-MAG_3 and ^{99m}Tc-DMSA, the mobility of ^{99m}Tc-MDP is significantly lower. It shows a rather broad peak in the electropherogram. This might be due to the not unambiguously defined complex structure by the existence of several formed ^{99m}Tc products. As another possibility, the coordinating hydroxyl group may be unstable and thus lead to a peak broadening. For all three complexes, no non-reacted $^{99m}TcO_4^-$ was found.

3.4. Conclusions

The capillary electrophoresis was successfully applied to a variety of clinically used ^{99m}Tc radiopharmaceuticals. The separation principle basing on the ratio of complex charge and molecular size proved to be very advantageous. The feasibility is, however, coupled to the availability of a reliably working radioactivity detection system with sufficient spatial resolution. The employed detection system showed a suitable sensitivity. Although it is not evident, the detection of single peaks is likely to indicate radiochemical purities of the complex preparations. In the analysis of both anionic and cationic complexes, the conventional CZE mode basing solely on the electroosmotic flow inside the capillary is not appropriate for all systems such as $^{99m}TcO_4^-$ or other electrophoretically fast anions. That could be overcome by the use of pressure-driven capillary zone electrophoresis.

Since only very small amounts of samples were used for CZE, no recovery could be determined. However, a factor that decreases the expected recovery of the radioactive signals of cationic complexes is a significant wall adsorption. This is exhibited by increased background levels for CZE of cationic complexes and can be assumed to be caused by electrostatic interactions between the negatively charged capillary wall and the positively charged complexes. Although wall adsorption could also be due to complex degradation during CZE, the detected peak shapes do not indicate labile complexes. However, a rapid desorption is advantageous. Furthermore, the buffer ionic strength could be increased and thus the wall interactions could be decreased. This is, however, connected with longer analysis times.

All investigated complexes showed expected electrophoretic behaviours. For the majority of the investigated complexes, the preparations appeared to be radiochemically pure. While for ^{99m}Tc-EHIDA and ^{99m}Tc-MDP no uniform complexes were expected, significant impurities in case of ^{99m}Tc-Q12 were obtained. That shows the potential of the CE method for the quality control of ^{99m}Tc complexes.

For neutral complexes, no detection time differences can be obtained. Possibly, the use of coupled electrophoretic-chromatographic methods, such as micellar electrokinetic capillary chromatography (MECC) or capillary electrochromatography (CEC) can deliver useful results. A decisive drawback of any insertion of chromatographic interactions into the electrophoretic system is the possible irreversible adsorption to the chromatographic matrix. Furthermore, the use of sodium dodecylsulfate (SDS) in MECC may possibly change the molecular structures of the complexes by transchelation effects.

In case of anionic ^{99m}Tc radiopharmaceuticals, only electrophoretically slow complexes could be detected using the conventional CZE mode. Fast anions were analyzed by application of the pressure-driven CZE mode.

To sum up, the CZE in connection with the pressure-driven CZE is appropriate for the analysis of ^{99m}Tc radiopharmaceuticals. It represents a quick and relatively cheap method for quality control in clinical routines as well as for radiopharmaceutical research. In part, the capillary electrophoresis is advantageous compared to chromatographic methods. However, analytical methods should always be used to complement it. Since CE devices connected with radioactivity detection systems are currently not commercially available, their development is desir-

able to make the method applicable for clinical routine.

References

[1] W.A. Volkert, S. Jurisson, Topics Curr. Chem. 176 (1996) 123.
[2] B. Johannsen, H. Spies, Topics Curr. Chem. 176 (1996) 77.
[3] S. Jurisson, D. Berning, W. Jia, D. Ma, Chem. Rev. 93 (1993) 1137.
[4] E. Deutsch, K. Libson, S. Jurisson, L.F. Lindoy, Progr. Inorg. Chem. 30 (1983) 75.
[5] M.J. Clarke, L. Podbielski, Coord. Chem. Rev. 77 (1987) 275.
[6] A.M. Verbruggen, Eur. J. Nucl. Med. 17 (1990) 346.
[7] F.A.A. de Jonge, E.K.J. Pauwels, Eur. J. Nucl. Med. 23 (1996) 336.
[8] K.D. Altria, Methods Mol. Biol. 52 (1996) 119–122.
[9] D. Kaniansky, P. Rajec, A. Svec, P. Havasy, F. Macasek, J. Chromatogr. 258 (1983) 238.
[10] G. Westerberg, H. Lundqvist, F. Kilar, B. Langstrom, J. Chromatogr. 645 (1993) 319.
[11] G. Westerberg, B. Langstrom, Appl. Rad. Isot. 45 (1994) 773.
[12] D. Kaniansky, P. Havasi, Trends Anal. Chem. 2 (1983) 197.
[13] K.D. Altria, C.F. Simpson, A.K. Bharij, A.E. Theobald, Electrophoresis 11 (1990) 732.
[14] G.C. Krijger, H.A. Claessens, H.T. Wolterbeek, Chem. Spec. Bioavail. 8 (1996) 29.
[15] A. Van Schedael, K. Verbeke, C. Van Neurom, J. Hoogmartens, A. Verbruggen, J. Chromatogr. B 697 (1997) 251–254.
[16] S.A. Faraq, C.E. Wells, Mikrochimica Acta 126 (1997) 141–145.
[17] R. Jankowsky, M. Friebe, H. Spies, B. Johannsen, J. Chromatogr. A, in press.
[18] R. Jankowsky, M. Friebe, German Patent Application No. 197 50 832.4.